Towards a more objective way of cervical cancer screening:

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2014

document version
Publisher's PDF, also known as Version of record

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1. EPIDEMIOLOGY OF CERVICAL CANCER

Cervical cancer accounts for 8.8% of all cancers in women worldwide, making it the third most common malignancy in women and the seventh overall[1]. With an estimated 530,000 new cases and 275,000 deaths in 2008, it is an important public health problem[1]. Overall, the mortality to incidence ratio of cervical cancer is 52%. However, the disease has a very uneven global distribution; over 85% of cases are found in low resource countries, with incidence and death rates being the highest in sub-Saharan Africa (age-standardised rates (ASR) > 30 per 100.000), Central America (ASR: 23.9 per 100.000), South-Central Asia (ASR: 24.6 per 100.000) and Melanesia (ASR 23.9: per 100.000 (Figure 1)[1-4]. This imbalance in disease burden can be explained by differences in background risk and the fact that cervical cancer is preventable by an effective screening and intervention system. Therefore, the lowest incidence and mortality rates are recorded in countries where screening is available to women, such as in the Netherlands.

In the Netherlands (16.8 million residents), approximately 2% of all newly diagnosed malignancies in women are cervical cancers, corresponding to about 700 new cases per year (age standardised incidence rate 6.0/100.000)[5;6]. Furthermore, every year about 200 women die from the disease (age-standardised mortality rate of 1.3/100.000), which is about 1.5% of all deaths in Dutch women caused by cancer [5;6].

2. HRHPV AND CERVICAL CARCINOGENESIS

2.1 Precursor lesions

Cervical cancer originates in the uterine cervix, which constitutes the lower part of the uterus that partly protrudes in the vagina (Figure 2). The cervix consists of two parts: the endocervix (the inside of the cervical canal) and the ectocervix (outer part of the cervix). The endocervix is lined by a single layer of glandular columnar epithelial cells, while the ectocervix and vagina are covered with squamous epithelium and is multi-layered. The border between these two types of epithelium is called the squamo-columnar junction (SCJ). From puberty onwards, vaginal PH acidification induces the replacement of a portion of endocervical columnar epithelium by a metaplastic squamous epithelium. The area between the original and the new SCJ is called the transformation zone (TZ). Until recently, the TZ was thought to be most susceptible to oncogenic influences, such as a transforming infection with high-risk types of the human papillomavirus (hrHPV). However, recent findings suggest that a small, discrete population of single layered, cuboidal epithelial cells of embryonic origin that localizes at the SCJ of the cervix, represents the likely cellular precursor of most cervical cancers and their precursor lesions[7].

Squamous cell carcinoma (SSC) is the most common histotype of cervical cancer (80%). The second most common type is adenocarcinoma (AdCa), accounting for approximately 15% of cervical cancers. The remaining 5% include rare types, such as neuro-endocrine carcinomas and clear-cell carcinomas.

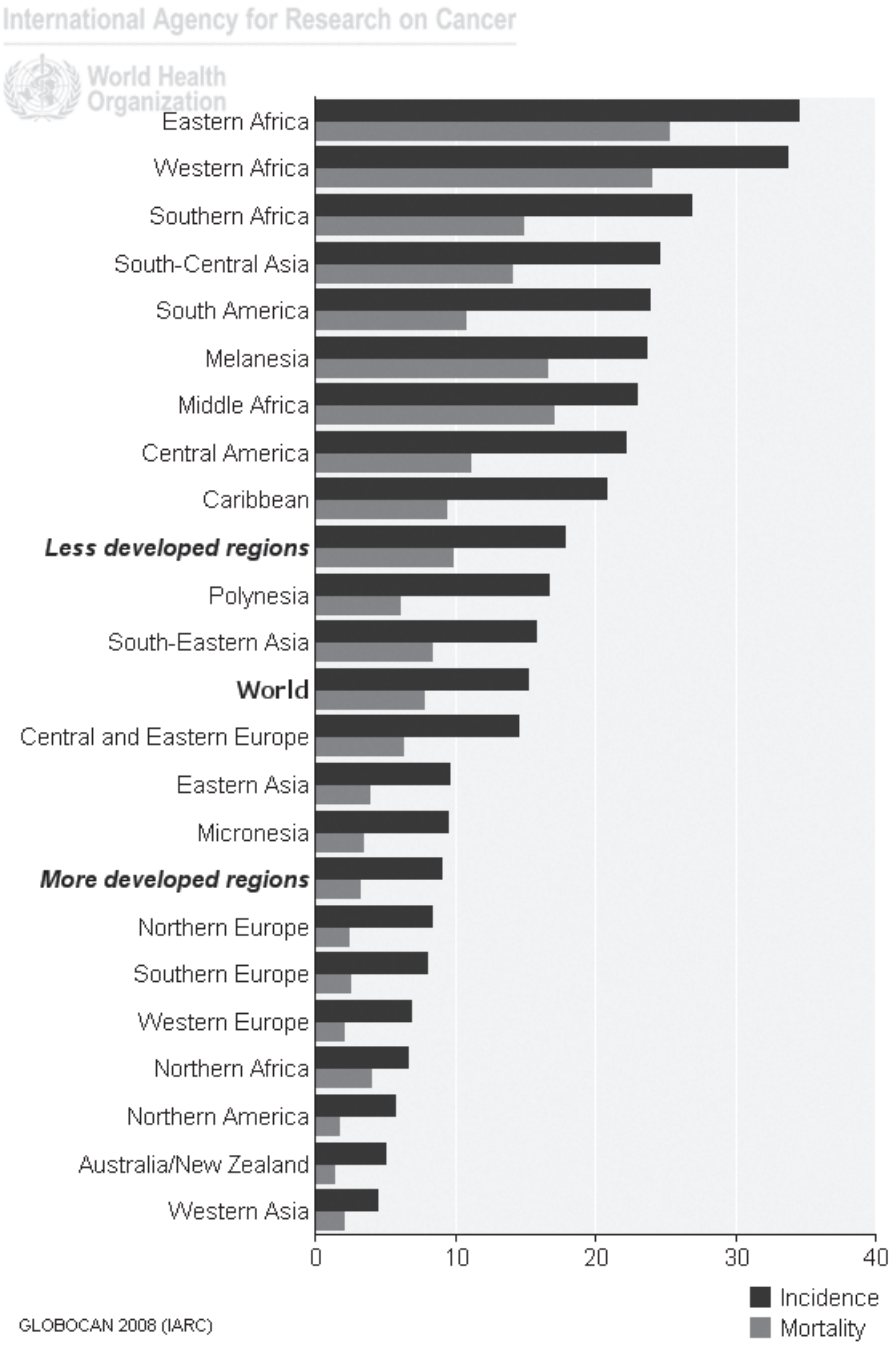

Figure 1. Cervical cancer incidence and mortality rates (age-standardised, world) per 100,000.

Cervical SSCs develop through well-characterised stages of premalignant lesions, so called *cervical intraepithelial neoplasia* (CIN), graded from 1 to 3 (CIN1, CIN2 and CIN3) [8]. In case of CIN1 (mild dysplasia) the lower 1/3 of the total thickness of the squamous epithelium shows atypia, in CIN2 (moderate dysplasia) the lower 2/3 of the epithelium is involved, and in CIN3 (severe dysplasia and carcinoma in situ) from 2/3 up to the whole epithelial layer consists of atypical cells. Different grades of dysplasia may coexist within the same cervix.

Accurate grading of CIN lesions is important for clinical management of patients, because CIN1 and CIN2/3 lesions are managed differently (i.e. surveillance versus radical treatment). Also, the outcome of cervical screening trials is dependent on accurate CIN lesion grading. However, histopathological diagnosis of CIN is complicated by a variety of cellular changes associated with inflammation, pregnancy and/or atrophy. These changes may mimic precancerous cervical lesions, thereby making cervical histology, that is, the diagnostic interpretation of H&E-stained slides, subjective and prone to variability [9]. This is reflected in poor inter-observer agreement between pathologists [10-12]. In particular, the differential diagnosis between immature squamous metaplasia and CIN1/2, or between low-grade (CIN1) and high-grade (CIN2/3) lesions, may be difficult [10;13;14]. To overcome these problems, doubtful lesions are usually judged by more than one pathologist, and in case of clinical trials the diagnosis of CIN lesions is often reviewed by expert pathologists. Collectively, this emphasizes the need for specific biomarkers to aid objective CIN lesion grading, and to identify true high-grade dysplasia of the cervix [15].

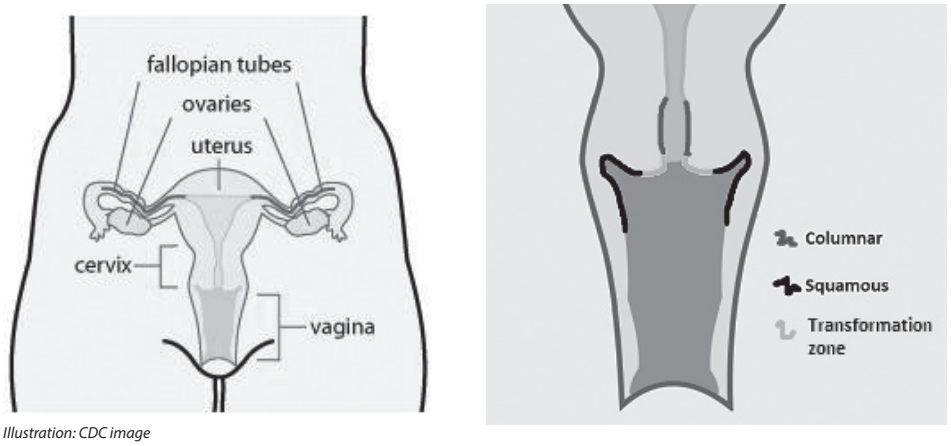

Illustration: CDC image

Figure 2. Anatomy of the uterus and cervix with transformation zone

2.2 Human papillomavirus

Mucosal human papillomaviruses (HPVs) comprise a common sexually transmitted virus family and the majority of both men and women are infected with such viruses shortly after starting sexual intercourse[16;17]. Papillomaviruses are small, double-stranded DNA viruses. To date there are over 170 different types of HPV identified, of which about 40 types are known to infect the genital mucosa[18]. It is assumed that the life-time risk of acquiring a genital HPV infection is at least 80% [19].

Since the 19th century it has already been recognised that cervical cancer is associated with sexual activity [20], however, it was not until the 1970s that the role of HPV in the development of cervical carcinoma was discovered by Harald zur Hausen and colleagues [21]. Since then, many studies have confirmed that persistent infection with a high-risk HPV (hrHPV) type is a necessary condition to develop cervical cancer and its premalignant lesions [22-25]. hrHPV can be detected in almost all cervical SSCs [22], and in 94 to 100% of all AdCas [26-28]. Low-risk (lr) HPV types such as HPV6 and HPV11 are associated with benign wart-like lesions. Based on epidemiological criteria at least a dozen hrHPV types have been identified (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) [24;26;29], and six types are considered as probably high-risk (26, 53, 66, 68, 73 and 82) [30]. Not all hrHPV types confer the same risk of high-grade cervical lesions. HPV16 causes more than half of the cervical cancers worldwide, followed by HPV18 (approximately 16%), and HPV33 (approximately 4%) [31]. hrHPV does not only play a causal role in the development of cervical cancer, but also in cancers of other parts of the anogenital tract, such as anal, vulvar, and penile cancer, as well as of the head and neck, particularly oropharyngeal cancer [30;32-35].

The HPV prevalence depends on geographic area, as discussed above, and age. Among women it is highest between the age of 20 and 25 years (approximately 20%) [17;36;37]. The prevalence gradually declines by increasing age to under 3% in women over 45 years of age [38]. Risk factors that are associated with acquiring an infection are the number of sexual partners, the age of sexarche, and smoking [25;39;40]. Fortunately, at least 80% of all hrHPV infections are transient, and will not result in premalignant lesions or cervical cancer. Most women clear the infection within 1-2 years after exposure [25;41]. In the remaining 20% the hrHPV infection persists, which is the first step towards the development of a premalignant lesion. However, additional (epi)genetic transforming events are necessary for malignant progression[25;42]. Thus, genital infections are very common, but only a few HPV-infected individuals (approximately 1-3%) ultimately show progression to invasive cancer [43].

2.3 HPV-mediated cervical carcinogenesis

HPV infections have a tight connection to the differentiation process of the infected epithelium. Besides the viral proteins E1 and E2, which are essential for viral replication, the virus relies entirely on the host cell DNA replication machinery to generate progeny. A productive HPV infection starts following entry of the virus into the basal cells of the cervical epithelium, and the virus replicates as these cells differentiate to produce the protective barrier normally provided by superficial epithelial cells. Viral E6 and E7 proteins are needed to allow vegative viral replication in differentiated, non-dividing epithelial host cells, in which the DNA replication machinery is normally not activated. The E7 protein binds to the retinoblastoma tumor suppressor protein (pRB) and disrupts the binding of pRB to E2F, which leads to S-phase entry through released E2F [44;45]. E6 binds to the human tumor suppressor protein p53 and degrades this protein, which is important to prevent cell-cycle blockade and apoptosis. When these cells disintegrate, as a consequence of their natural turnover at the superficial layers, new formed, infectious viral particles are released into the environment [42;46]. Thus, the E6 and E7 proteins are necessary for the virus to replicate itself and are expressed during the productive "normal" life

cycle, where their regulation is tightly controlled. This productive infection may give rise to mild or moderate cellular abnormalities, histologically comparable with CIN1/2, but not often to true pre-malignant cervical lesions.

Alternatively, a so-called persistent transforming HPV infection may arise, under conditions that the viral E6 and E7 proteins are improperly expressed in the proliferating basal cells, stimulating viral transformation [47;48]. These transforming infections are associated with CIN2, CIN3 and cervical cancer. The mechanism underlying the deregulated expression of E6 and E7 is not fully understood. A possible explanation is integration of viral DNA in the host cell genome [49;50], but methylation of E2 binding sites has also been suggested [51]. The constant over-expression of viral proteins E6 and E7 will result in chromosomal instability and provides the driving force to further progression towards cancer. The process is as follows: free E2F resulting from the interaction of E7 with pRB stimulates uncontrolled cell growth in proliferating cells [52]. Subsequently, the cell cannot cope with the uncontrolled growth, due to degradation of tumor suppressor protein p53 by E6, which triggers the development of genetic instability [53]. In addition, as a consequence of deregulated expression of E7, the tumor suppressor protein $p16^{INK4a}$ is up-regulated. $p16^{INK4a}$ is a cyclin dependent kinase inhibitor that normally prevents inactivation of pRB by cyclin D1 and therefore induces cell cycle arrest at G1. However, in the presence of hrHPV E7 the pRB protein is already inactivated, and thus upregulated $p16^{INK4a}$ has no effect. Yet, the overexpression of $p16^{INK4a}$ throughout the cervical epithelium can be considered as a marker for lesions that harbour a transforming hrHPV infection [54-56].

In conclusion, cervical cancer develops through the following steps: hrHPV infection, hrHPV persistence, hrHPV transformation and development of premalignant lesions, and finally progression to invasive cervical cancer (Figure 3) [25]. Although the process between first hrHPV infection and evidence of a premalignant lesion usually requires many years, time can be as short as 2 to 3 years [36;42;57]. Furthermore, only 1-3% of all hrHPV positive women will develop a transforming infection [26], which could ultimately result in invasive cancer [42;58]. Next to persistence of a transforming infection, accumulation of additive (epi)genetic alterations is necessary for further progression towards invasive cancer. This final step takes on average 15-20 years [42;59]. As mentioned earlier, a discrete population of embryonic epithelial cells represent the likely cellular precursor of most cervical cancers and their precursor lesions [7]. Apparently, these cells, which are characterized by a specific immunostaining pattern, are highly susceptible to transformation by hrHPV. Conversely, productive infections are supposed to arise exclusively from infection of basal cells of the squamous epithelium lining the ectocervix or adjacent transformation zone [60].

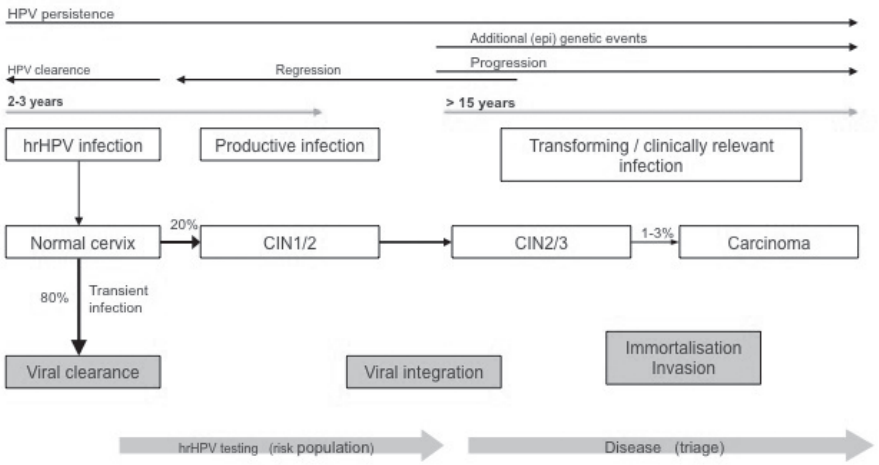

Figure 3. Progression model of cervical cancer [Adapted from: Snijders et al. 2006] [42] .

3. PREVENTION OF CERVICAL CANCER

Primary prevention seeks to prevent, by interventions, the onset of specific diseases via risk reduction, thus prevention of disease in healthy people. This can be achieved by altering behaviours or exposures that can lead to disease, or by enhancing resistance to the effects of exposure to a disease agent. In cervical cancer prevention, prophylactic vaccination with HPV16/18 L1 virus-like particles (VLPs) is an example of primary cancer prevention. VLPs resemble the actual virus morphologically but cannot induce infection, as these do not contain virus DNA.

Secondary prevention comprises procedures that detect and treat pre-clinical non-symptomatic pathological changes and thereby control disease progression. Cervical cancer screening by detection of abnormal cells in a cervical smear sample (Pap smear) is an example of secondary cancer prevention.

3.1 Primary cervical cancer prevention

In the Netherlands, two prophylactic vaccines are available since 2009. A bivalent HPV16/18 L1 VLP vaccine that protects against HPV16 and HPV18 (Cervarix®, GSK), and a quadrivalent vaccine that also contains VLPs of non-oncogenic types HPV6 and HPV11 (Gardasil®, MSD) [61-66]. These vaccines provide protection against persistent infection of epithelial cells with HPV types represented in the vaccine, and against incident and persistent CIN2 or worse (CIN2+) lesions caused by the vaccine HPV types. In principle the protection is type-specific, but cross-reactivity against HPV45, and partially against HPV31 has been proven [28]. At present, however, vaccination does not eliminate the need for screening, as the HPV vaccines only protect against HPV types

which together cause about 77% of cervical cancer cases (i.e., 70% by HPV16/18, 7% by cross-protection) [28;67]. In addition, the vaccine uptake remains suboptimal in the Netherlands, where HPV vaccination is offered to HPV-negative (naïve) 12-year-old girls, and an average of 56% coverage is observed [68]. Consequently, secondary cancer prevention, i.e., screening, will remain the most important preventive strategy at least for the next two decades.

3.2 Secondary cervical cancer prevention

The fact that cervical cancer develops through different premalignant stages (precursor lesions) which can be detected years before cervical cancer appears [25;42], offers possibilities for screening and treatment. This recognition has resulted in the organisation of population-based screening programs. The benefit of such a screening program depends on the level of participation of invited women, the clinical sensitivity of the screening test, availability of an adequate intervention, and adequate follow-up protocol for women with an abnormal test result [25]. Clinical sensitivity is the probability that a test correctly identifies people with clinically meaningful disease at a preclinical stage as positive.

However, there are also negative effects of screening, such as distress due to a false positive screening test, and the risk of unnecessary treatment and possible adverse effects of surgical treatment.

3.2.1 Current cervical cancer screening

The cytological smear, also known as Pap smear, is based on cytomorphological examination of exfoliated cells from the transformation zone, cells from the ectocervix and cells from the endocervical epithelium [69]. The Pap smear is named after Dr. Papanicolaou who was the first to demonstrate that (precursor lesions of) cervical cancer could be detected by this method [70]. This finding resulted in the worldwide implementation of cytology-based screening as a diagnostic tool to identify cervical disease.

In the Netherlands, cervical cancer screening using the Pap smear was introduced in the beginning of 1970s for women between the age of 35-55 years, with a smear taken every 3 years. In 1996, the screening program was revised and changed into a program with a 5-year interval for women aged 30-60 years [71]. The main goal was to increase the effectiveness and to decrease the number of opportunistic smears taken outside the screening program. Another important change in the restructuring was implementation of a new follow-up algorithm combined with a more consistent classification of the cytological smear result (CISOE-A classification) [71;72]. This resulted in a decrease in the number of equivocal diagnoses from 11.3% in 1990 to 2.6% in 2000 ($p < 0.001$) [73]. The CISOE-A classification (in Dutch KOPAC-B) can be converted into the (internationally used) Bethesda system (Table 1) [73;74]. The new follow-up algorithm implied that, in case of borderline or mild dyskaryosis (BMD), women were recalled for repeat cytology testing after 6 and 18 months and were referred for colposcopy if the repeat test result was positive (BMD or worse) at either of these occasions. This policy is used because only 5-15% of women with BMD has or will develop high-grade precursor lesions [75-77].

Table 1. Classification systems to report cervical cytology

A: Squamous

Classification to report cervical cytology: squamous

Description	Normal	Borderline	Mild dyskaryosis	Moderate dyskaryosis	Severe dyskaryosis or CIS	Malignant
KOPAC-B * (CISOE-A)	nnPAC:	nnPAC:	nnPAC:	nnPAC:	nnPAC:	nnPAC:
	nn1[1-2][1-2]	nn[2-3][1-2][1-2]	nn4[1-2][1-2]	nn5[1-2][1-2]	nn6[1-2][1-2]	nn[8-9][1-2][1-2]
NHS CSP	Negative	Borderline change in squamous cells	Low-grade dyskaryosis	High-grade dyskaryosis (moderate)	High-grade dyskaryosis (severe)	High-grade dyskaryosis (severe) / ?invasive squamous carcinoma
Bethesda 2001	NILM, Atrophy	ASC-US / ASC-H	ASC-H / LSIL	HSIL	HSIL**	SSC
Managment	Next invitation	Follow-up testing	Follow-up testing	Referral for colposcopy	Referral for colposcopy	Referral for treatment

*KOPAC-B formally introduced for cervixcytology in 1996 (with continued use of pap translation)

**if invasion is suspected, add: "with features suspicious of invasion"

B: Glandular (endocervical)

Classification to report cervical cytology: glandular (endocervical)

Description	Normal	Borderline	Mild dyskaryosis	Moderate dyskaryosis	Severe dyskaryosis or AIS	Malignant
KOPAC-B # (CISOE-A)	nnPAC: nn1[1-2][1-2]	nnPAC: nn1[1-3][1-3]	nnPAC: nn1[1-2,4][1-2,4]	nnPAC: nn1[1-2,5][1-2,5]	nnPAC: nn1[1-2,6][1-2,6,7]	nnPAC: nn1[1-2,7,8][1-2,9]
NHS CSP	Negative	Borderline change in endocervical cells	Low-grade dyskaryosis	High-grade dyskaryosis (moderate)	High-grade dyskaryosis (severe)	?Glandular neoplasia of endocervical type / ?Glandular neoplasia (non-cervical)
Bethesda 2001	NILM, Atrophy	NOS and specify endocervical, endometrial or glandular	AGC favor neoplasia	AGC favor neoplasia	AIS, specify if endocervical adenocarcinoma in situ	AC : endocerv, endomet, glandular or other neoplasm
Managment	Next invitation	Follow-up testing	endocerv: follow-up endomet: colposcopy	Referral for colposcopy	Referral for colposcopy	Referral for colposcopy

Double scores are posisible, e.g. 514: moderate squamous dysplasia & mild endocervical dysplasia
example: C3 = atypical endocervical cells

NILM, negative for intraepithelial lesions or malignancy; ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous intraepithelial lesion cannot exclude HSIL; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; AGC, atypical glandular cells; AIS, endocervical adenocarcinoma in situ; SSC, squamous cell carcinoma; AC, adenocarcinoma.

In the present day, approximately 800,000 women are invited annually for cervical screening in the Netherlands [78]. On average, 65% of the invited population participates in screening, and another 12% are screened on indication or have opportunistic screening. About 95% of these participants has a normal smear, and these women are invited for the next screening round in 5 years. Around 7000 women need re-testing because the sample is of inadequate quality, and BMD is found in approximately 2-2.5% of smears, followed by the advice to repeat testing. Over 3500 women are referred for colposcopy immediately because their pap smear reveals moderate dyskaryosis or worse [79;80]. Finally, each year more than 5000 women are treated for a high-grade CIN lesion to prevent the development of cervical cancer, mainly through a loop electrosurgical excision procedure (LEEP) [81].

Several epidemiological studies have shown that cytological screening, as implemented in the Netherlands, has been successful in reducing the incidence and mortality of cervical cancer [82-85]. However, cervical cancer continues to be a considerable public health problem [86;87]. The main reasons for missing cervical cancer cases despite a screening program are a relatively low attendance rate (65% of invited women) and a relatively high number of false-negative cytology tests, due to the rather low sensitivity (50-70%) of the pap smear [88;89]. Further, cytology has low reproducibility, leading to variable accuracy [90;91]. Moreover, by repeating cytology, the number of false-positives increases substantially over time [92]. In addition, cytology is a subjective test and labour intensive [93]. Finally, the decrease in incidence of cervical cancer induced by cytology-based screening is mainly restricted to squamous cell carcinoma, whereas no change is observed in incidence of cervical adenocarcinoma [94-96], suggesting that cytology fails to detect adenocarcinomas and its precursors. Therefore, over the last decades, efforts to improve cervical screening have focused on increasing adherence to the screening program and on development of alternative (objective) screening tests that are more sensitive than cytology.

4. IMPROVEMENT OF CERVICAL CANCER SCREENING

4.1 hrHPV-based cervical cancer screening

The causal relationship between infection with hrHPV and cervical cancer has stimulated the application of hrHPV DNA testing, which has been proposed, either alone or in combination with cytology, as a means to improve existing cervical screening programs. In the past fifteen years, large randomized trials designed to evaluate the performance of hrHPV testing, have provided important arguments for the implementation of this assay as a primary screening tool [97-106].

These studies have shown that hrHPV testing detects 30% more CIN2+, and 20% more CIN3 or worse (CIN3+) lesions in women over 30 years of age. In addition, four randomized trials conducted in Europe, have published longitudinal data on CIN3+ diagnosed at subsequent screening rounds, which took place in 3 to 5 years [98;100;101;104;107]. All trials reported an approximately 50% lower CIN3+ detection rate in the second screening round among women who were hrHPV negative at baseline, than among women who had normal cytology. Most importantly, in a

pooled analysis of these European trials, it was recently confirmed that hrHPV-based screening provides better protection against *cervical cancer* than cytology [108]. Thus, a negative hrHPV test provides a better protection against high-grade precursor lesions and cervical cancer than a negative Pap smear, permitting less frequent screening.

The HPV test used in these trials were the hybrid capture 2 (HC2) test and the GP5+-6+-PCR EIA assay, which are considered clinically validated for screening purposes [109;110]. The hrHPV HC2 is a signal amplification method in a liquid-phase format and uses a mixture of full length RNA probes representing 13 HPV types (i.e., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) to hybridize to HPV DNA in heat-alkaline-denatured samples. RNA/DNA hybrids are detected by peroxidase-labeled antibodies and visualized by electro-chemiluminescence (ECL) [111]. The GP5+-6+-PCR EIA test is a PCR-based assay, amplifying L1 DNA of a broad-spectrum of HPV types. The read-out involves an enzyme immunoassay (EIA) staining procedure using oligoprobe cocktails representing high-risk and/or low-risk HPV types [112]. However, many new hrHPV tests have been developed, and the clinical performance of these tests is mostly unknown. Therefore, standards for hrHPV test performance and characteristics in clinical practice have been formulated by an international consortium [109]. Thus far, several additional hrHPV DNA tests, such as Cobas 4800 Roche®, RealTime (RT) PCR Abbott Molecular®, and Papillocheck Bio-Greiner® (when only 14 hrHPV types are considered), have fulfilled the criteria provided in these guidelines, and can be considered clinically validated for primary hrHPV-based cervical cancer screening [113-119].

Another important and consistent finding in trials with combined hrHPV and cytology co-testing, is that co-testing has virtually no additional value compared to single hrHPV screening [120-122].

Collectively, the available evidence indicates that sole hrHPV testing should replace cytology as a primary screening tool in cervical screening. Therefore, the Dutch Minister of health has recently decided to use primary hrHPV screening to improve cervical screening efficacy. This will be implemented in the Netherlands in 2016 and comprises primary hrHPV testing in 5 screening rounds, at the ages of 30, 35, 40, 50 and 60 years [123].

4.2 Triage of hrHPV positive women

The drawback of primary hrHPV testing is that it has an (apparently) unavoidable trade-off between sensitivity and specificity. Overall, hrHPV testing has a 3-4% lower specificity for high-grade CIN than cytology [122]; the hrHPV test detects a substantial number of women with transient hrHPV infections that will not lead to clinically meaningful lesions [124;125]. This may lead to unnecessary referral for colposcopy and possible over-treatment [103]. As a result, triage testing of hrHPV positive women is necessary to keep the number of invasive follow-up examinations, and associated costs within acceptable limits [126].

Epidemiological studies have indicated that detection of HPV16, HPV18, or both might be used to identify women with an increased risk for CIN3+ [107;121;127]. Yet, in a recent hrHPV screening trial [126], direct triage with cytology and repeat cytology testing at 12 months emerged as a suitable

implementation strategy. Thus, there is no consensus on the best way to manage hrHPV DNA positive women. In addition, in future screening, it is likely that the role of cytology becomes more limited and validated (molecular) biomarkers gain attention; among these, $p16^{INK4a}$/Ki-67 dual staining and host genome or viral DNA methylation markers appear to be promising [128-132].

4.3 Increasing screening coverage

One other problem concerning the effectiveness of current cervical screening programs remains non-attendance [133-136]. Non-participating women are at increased risk of cancer [82;137], as half of the cervical carcinomas are found in these women. Therefore, targeting non-attendees is important in achieving optimal protection from screening programs. Self-sampling is a less costly and less invasive collection method [138], and several studies have shown that non-attendees actually do take part in self-sampling studies [133-135;139-143]. Moreover, the detection of high-grade lesions in non-attendees participating in self-sampling was higher than in regular screening attendees [134;142;144]. Studies using interview surveys have shown that women prefer self-collection over physician-collection [138;145;146]. Thus, there is a basis for self-sampling in cervical cancer screening. In addition, self-collection makes cervical screening accessible to women in medium- and low income countries [136;147;148]. That is why in recent years several studies have focused on the use of self-collected samples for hrHPV testing. Studies have shown that hrHPV testing on self-samples can be non-inferior to that of physician-collected cervical samples for the detection of CIN2+, although reported data are rather inconsistent [138;140;149-151]. This most likely reflects the use of different self-collection devices in combination with different HPV tests [151;152]. Therefore, it is important that a self-collection device is clinically validated in combination with a hrHPV test, prior to its use as a hrHPV self-sampling procedure to re-attract non-attendees in population-based screening, or even for primary hrHPV-based cervical screening.

5. AIM AND OUTLINE OF THIS THESIS

Cervical cancer is induced by a persistent infection with hrHPV. This causal link has been proven indisputably, and strong evidence now supports the use of hrHPV testing in the prevention of cervical cancer. The aim of this thesis was to evaluate the clinical accuracy of hrHPV selfsampling, the value of different triage strategies and biomarker-guided CIN grading. More specifically, the studies described in this thesis aimed to answer the following questions:

- Is brush-based self-sampling in combination with GP5+/6+-PCR EIA hrHPV testing valid for assessing CIN2+ risk, in comparison to hrHPV testing on physician-taken scrapes? And what is the compatibility between self- and physician-collected samples at the level of HPV genotyping? (**Chapter 2**: Brush-based self-sampling in combination with GP5+/6+-PCR-based hrHPV testing: High concordance with physician-taken cervical scrapes for HPV genotyping and detection of high-grade CIN)

- Does replacing the first generation self-sampling lavage device by an ergonomically improved, second generation device result in higher response rates among non-responders of the Dutch cervical screening program? And is the clinical performance of both models comparable? (**Chapter 3**: A second generation cervico-vaginal lavage device shows similar performance as its preceding version with respect to DNA yield and HPV DNA results)
- What are implementable triage strategies for primary hrHPV screening in an organized setting? (**Chapter 4**: Primary hrHPV DNA testing in cervical cancer screening: how to manage screen positive women? A POBASCAM Trial sub study)
- What are benefits of hrHPV-based screening compared to cytology-based screening. And what is the duration of the protection provided by a negative triage test for future CIN3 and cancer, for several strategies to triage hrHPV positive women.(**Chapter 5**: CIN3 and cancer risks after primary HPV DNA testing and cytology triage in cervical cancer screening: fifteen years follow-up of a randomized controlled trial)
- Does the use of $p16^{INK4a}$ immunohistochemistry improve the accuracy of grading CIN lesions? (**Chapter 6**: $p16^{INK4a}$ immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions)
- In **Chapter 7**, we provide an overview of the arguments in favour of, and concerns on aspects of implementation of hrHPV testing in primary cervical cancer screening (discussion). This thesis ends with a summary of the findings, and future prospects in **Chapter 8**.

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